7. Nitrostyrene Derivatives of Adenosine 5′-Glutarates Modified with an Alkyl Spacer and Their Inhibitory Activity on Epidermal Growth Factor Receptor Protein Tyrosine Kinase

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β-Nitrostyrene derivatives of adenosine 5′-glutarates are potent and selective bisubstrate-type inhibitors of the epidermal growth factor receptor protein tyrosine kinase (EGF-R PTK). In an attempt to improve the inhibitory activity, this type of compounds was modified with alkyl spacers of varying length between the nitrostyrene and the glutaryl units. The spacers consisted of 1, 3, 4, and 5 atoms to give compounds of the benzyl, oxyethyl, oxypropyl, and oxybutyl series, respectively (*Schemes 1* and *2*). Adenosine 5′-esters were prepared in the benzyl and oxypropyl series only. Compared to the compounds in the parent series without spacer ($IC_{50} = 0.7$–12 μM), most of the modified compounds inhibited the EGF-R PTK only marginally or were inactive ($IC_{50} \geq 100$ μM). The only exceptions were the free acids **19** and **20** with IC_{50} values of *ca.* 5 μM. It is noteworthy that esterification of these two hydrogen glutarates with either MeOH or adenosine yielded inactive compounds, which is in contrast to the corresponding substances without spacers.

Introduction. – Cell proliferation is tightly regulated by growth factors and hormones constituting growth signals. Growth signals are transmitted across the cell membrane to the nucleus *via* a signal-transduction pathway. This applies to the proliferation of normal as well as malignant cells, whose growth is out of control. In recent years, broad knowledge about these signalling events converting a normal cell to a malignant cell has been accumulated. In the response of cells to such regulatory signals, phosphorylation of proteins on tyrosine residues by protein tyrosine kinases (PTK's) is of prime importance.

Among the best understood members of the protein tyrosine kinase families are the growth-factor receptor PTK's [3–8] such as the epidermal growth factor receptor (EGF-R), platelet-derived growth factor receptor (PDGF-R), or insulin-like growth factor receptor (IGF-R) PTK's. The EGF receptor is a transmembrane glycoprotein that mediates the mitogenic response of cells to the epidermal growth factor (EGF) and the transforming growth factor-α (TGF-α) [4] [8]. The receptor consists of an extracellular hormone-binding domain, a short hydrophobic transmembrane domain, and an intracellular region which contains a ligand-activatable protein tyrosine kinase domain [3] [4] [8]. Enzymatic activity of the kinase domain is essential for signal transduction *via* the EGF-R [9] [10]. The EGF-R is involved in epithelial proliferation and has strongly been

[1]) From the Ph. D. thesis of *St. P.* [1].

[2]) From the diploma thesis of *D. H.* [2].

implicated in malignant tumor growth (*e.g.* breast tumors) [3]. The role of the EGF-R PTK in epithelial proliferation suggests that selective enzyme inhibitors could have therapeutical potential in the treatment of malignant and nonmalignant epithelial diseases. Due to the involvement of PTK's in many signal-transduction pathways, it will be important to develop agents with high enzyme selectivity. In our study, we have selected the EGF-R PTK as the target for the rational design of PTK inhibitors.

Concept and Design of Inhibitors. – In general, protein tyrosine kinases catalyze the direct transfer of the γ-phosphate group of ATP to a tyrosine moiety in a substrate peptide molecule. For this transfer, a transition-state was postulated with a pentacoordinated γ-P-atom and with bivalent metal ions, usually Mg^{2+} or Mn^{2+}, forming a complex with the α-, β-, and γ-phosphate groups to hold ATP in an optimal conformation for the phosphate transfer (*Fig.*) [11] [12]. Based on this transition-state, multisubstrate-complex analogues were designed that contained a β-nitrostyrene part as a tyrosine mimic, the sulfonylbenzoyl or the glutaryl (= pentanedioyl) moiety as the triphosphate substitute, and adenosine as nucleoside [13] [14]. The thus obtained transition-state analogues were highly potent bisubstrate-type inhibitors of the EGF-R PTK ($IC_{50} = 0.7$ μM) and showed selectivity with respect to other PTK's such as *c-src* or *v-abl* as well as to serine kinases such as protein kinase C (PKC) or cyclic AMP-dependent protein kinase (PKA) [14].

When we started our work using the glutaryl moiety as a triphosphate substitute [14], no crystal structure data on either a serine or a tyrosine kinase were available. The model

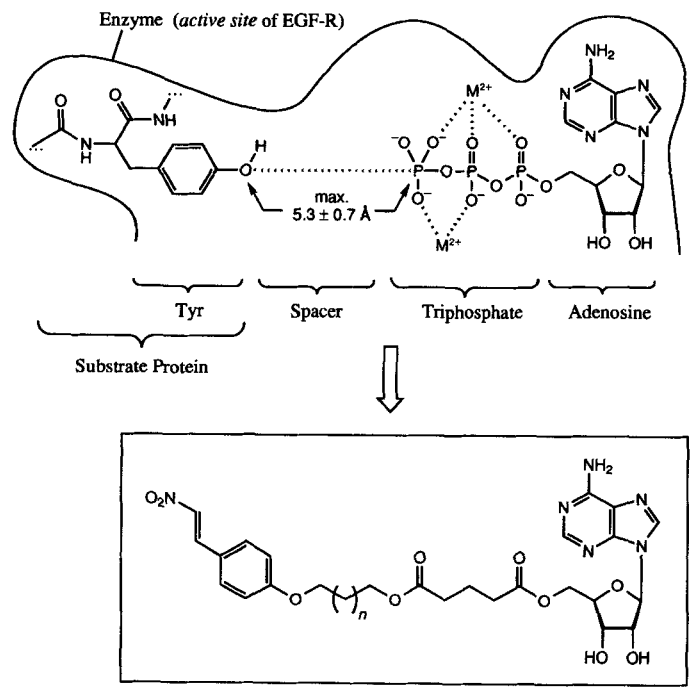
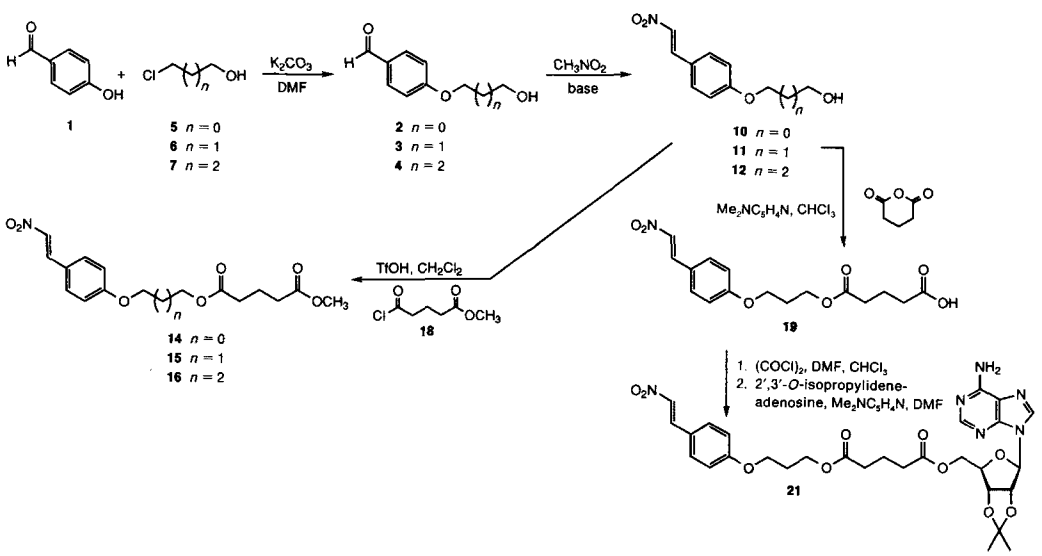


Figure. *Postulated transition state* (adapted from [12]) *and schematic representation of possible inhibitors*

we used was derived from NMR NOE measurements using the protein kinase PKA. In this model, *Kaiser* and coworkers [12] postulated a distance between the serine O-atom and the γ-P of 5.3 ± 0.7 Å in the transition state. Since it is known that there is high homology within the amino-acid sequences of the catalytic domains of all protein kinases [15], we assumed that during the catalytic process in a protein tyrosine kinase, a similar distance might occur between the tyrosine O-atom and the γ-phosphate of ATP. We, therefore, postulate a modified transition-state for the phosphorylation of tyrosine-containing substrate peptides by the EGF-R PTK (*Fig.*). Based on our previous work and this modified transition-state, we designed several series of transition-state analogues with a β-nitrostyrene moiety as the tyrosine mimic, an aliphatic spacer of varying length, the glutaryl moiety as triphosphate substitute, and adenosine as nucleoside. Using 2-chloroethanol, 3-chloropropanol, and 4-chlorobutanol, we synthesized compounds of the corresponding oxyethyl, oxypropyl, and oxybutyl series, which contain 3, 4, and 5 spacer atoms, respectively. For the inhibitor series with one spacer atom, β-nitrostyrene with a 4-hydroxymethyl group was used as the tyrosine mimic.

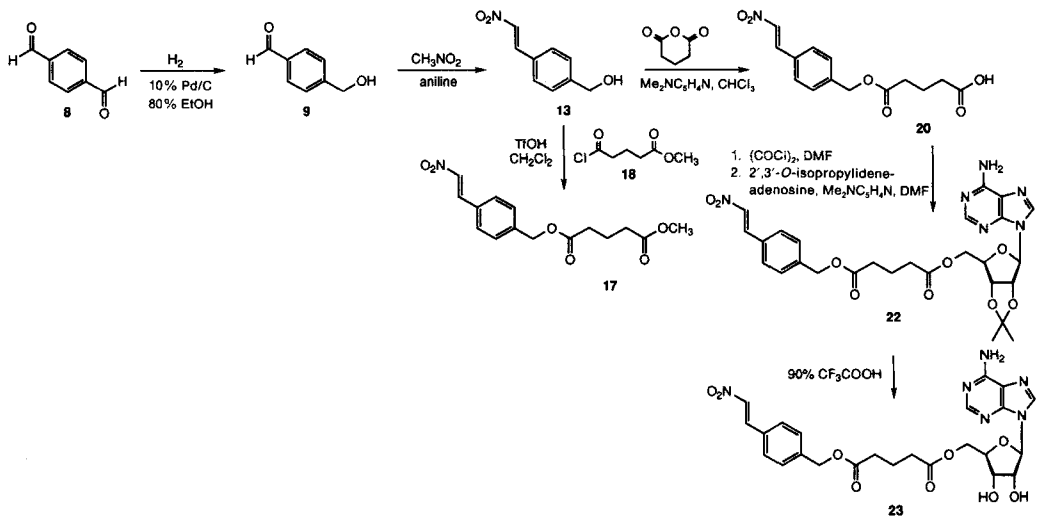
Syntheses. – Starting from 4-hydroxybenzaldehyde (**1**), the chain-elongated aldehydes **2–4** were prepared by base-catalyzed reaction with 2-chloroethanol (**5**), 3-chloropropanol (**6**), and 4-chlorobutanol (**7**), respectively, following a procedure by *Stoddart* and coworkers [16] (*Scheme 1*). Starting from benzene-1,4-dicarbaldehyde (**8**),

Scheme 1



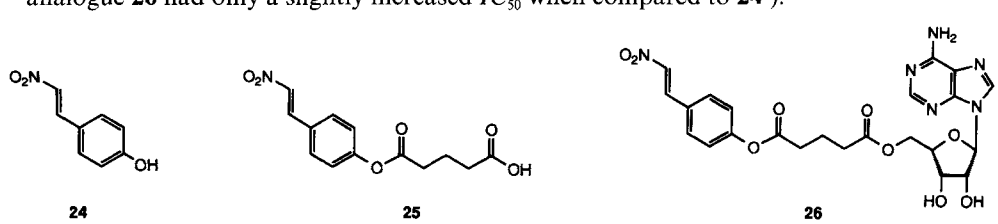
4-(hydroxymethyl)benzaldehyde (**9**) was synthesized by catalytic reduction over Pd/C according to [17] in 74% yield (*Scheme 2*). The aldehydes **2–4** and **9** were converted to the corresponding nitrostyrenes **10–13** by a nitro-aldol condensation; $MeNH_2$ was the catalyst in the transformation of **3** in analogy to [18], whereas aniline was used with **2**, **4**, and **9** [14]. The tyrosine-triphosphate analogues **14–17** could be obtained by reaction of the corresponding nitrostyrenes **10–13**, with methyl 5-chloro-5-oxopentanoate (**18**) and tri-

Scheme 2



fluoromethanesulfonic acid (TfOH) as catalyst [14]. The free acids **19** and **20** were synthesized by reaction of the corresponding nitrostyrenes **11** and **13** with glutaric anhydride following our previously published procedure [14]. The tyrosine-triphosphate analogues **19** and **20** were converted to their acyl chlorides by the mild oxalyl chloride/DMF method and then reacted *in situ* with 2′,3′-*O*-isopropylideneadenosine to yield the 5′-adenosyl esters **21** and **22**, respectively, which represent protected complete transition-state analogues. In addition, the isopropylidene group of **22** was removed with 90% CF_3COOH to give the deprotected compound **23**.

Results and Discussion. – The nitrostyrene and glutaryl-nitrostyrene derivatives **11**, **13–17**, and **19–23** were tested for inhibition of the EGF-R PTK using a purified recombinant intracellular domain of the EGF-R (EGF-R ICD) [19] as enzyme source and angiotensin II as the phosphoryl acceptor substrate (*Table*). The *β*-nitrostyrene derivative **24** used as tyrosine mimic was found to be a moderately potent inhibitor of the EGF-R PTK [13]. No significant increase of kinase inhibition was observed when the glutaryl moiety was added to the nitrostyrene unit [14]. Thus, the acid **25** showed an IC_{50} value comparable to that of the parent compound **24** [14]. Even the transition-state analogue **26** had only a slightly increased IC_{50} when compared to **24**[3]).



24 **25** **26**

[3]) In our previous report [14], crude A431 membranes were used as the enzyme source for the EGF-R PTK. In that former assay system, the transition-state analogue **26** was *ca.* 10 times more active than the nitrostyrene **24**.

Table. *Inhibitory Activities of Compounds* **11**, **13–17**, *and* **19–26**[a])

	Inhibition of protein kinases, IC_{50} [μM]			
	EGF-R ICD	*v-abl*	*c-src*	PKCα
11	4.4	> 100	> 100	46
13	60	> 100	> 100	n.t.
14	> 100	n.t.	n.t.	n.t.
15	> 100	n.t.	n.t.	n.t.
16	> 100	n.t.	n.t.	n.t.
17	> 100	n.t.	n.t.	n.t.
19	5.5	> 100	> 100	30
20	3.6	> 100	60	> 100
21	> 50	n.t.	n.t.	n.t.
22	> 50	n.t.	n.t.	n.t.
23	81	> 100	75	n.t.
24	6.9	> 100	> 100	> 100
25	6.1	> 100	> 100	37
26	2.9	43	20	52
Genistein	1.9	10	75	15
Erbstatin	9.6	> 100	> 100	> 100

[a]) n.t.: not tested.

Using alkyl spacers of varying length between the nitrostyrene moiety and the glutaryl part, no increase of activity could be observed: In the oxypropyl series, the nitrostyrene **11** and the acid **19** were similarly active as **24** or **25**. However, the 2′,3′-*O*-isopropylidene-adenosine 5′-ester **21** was inactive. In the oxyethyl, oxypropyl, and oxybutyl series, the methyl esters **14–16** were also inactive. In the benzyl series, only the free acid **20** was active ($IC_{50} = 3.6$ μM); all the other compounds, including the parent 4-(hydroxymethyl)-β-nitrostyrene **13** and the protected adenosine 5′-ester **22**, as well as the deprotected derivative **23**, were inactive ($IC_{50} > 50$ μM).

With respect to selectivity, active compounds of this series (**11**, **19**, and **20**) were inactive or only marginally active against other PTK's (*c-src* or *v-abl*) or serine kinases such as PKC_{α}.

The results presented here indicate that the insertion of an alkyl spacer unit between the β-nitrostyrene and the glutaryl moiety does not improve the inhibitory activity of nitrostyrene derivatives of adenosine 5′-glutarates against the EGF-R tyrosine kinase. On the contrary, a decrease or even complete loss of activity is observed for most compounds. Specifically, esterification of the hydrogen glutarates **19** and **20** with either MeOH or adenosine results in compounds which show only marginal activity or are totally inactive as inhibitors of the EGF-R tyrosine kinase. In view of some recent crystallographic data on protein kinases, these findings are not so surprising. In fact, the first X-ray structure of protein kinase, namely of the catalytic subunit of *c*-AMP-dependent protein kinase (PKA) complexed with Mg-ATP and an inhibitor peptide at 2.7 Å resolution, was recently reported [20] [21]. It shows that the distance between a serine O-atom and the γ-phosphate has to be revised to *ca.* 3 Å in the transistion state instead of the 5.3 ± 0.7 Å stated by *Kaiser* and coworkers [12].

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Experimental Part

General. All chemicals were purchased from *Fluka AG* or *Merck GmbH* in *purum* or *puriss.p.a.* quality. Solvents used in reactions were distilled and dried. Solvents used for column chromatography (industrial grade) were distilled once. $CHCl_3$ and CH_2Cl_2 were passed through a 15-g column filled with glass wool, neutral aluminium oxide, molecular sieve (3 Å and 4 Å, activated in microwave oven), and sand to remove H_2O and stabilizer (EtOH). Org. extracts were dried (Na_2SO_4), evaporated on a rotary evaporator ($\leq 30°/\geq 15$ mbar) and dried under high vacuum (≥ 0.1 mbar). TLC: *Merck* precoated glass plates, 0.25 mm silica gel *60* F_{254}, detection in UV after exposure to I_2 vapors. Flash chromatography (FC): columns with H_2O cooling; overpressure *ca.* 0.3 bar, *Merck* silica gel *60*, 40–63 μm, or *Merck Florisil*, 300–400 mesh. M.p.: *Kofler* hot stage with polarizing filters; uncorrected. UV: 1-cm or 1-mm quartz cuvettes; *Perkin-Elmer Lambda 9*. FT-IR: KBr pellets; *Perkin-Elmer 1600*. NMR: *Varian VXR-400* (^{1}H: 400 MHz; ^{13}C: 101 MHz), *Varian Gemini-300* (^{1}H: 300 MHz; ^{13}C: 75 MHz); δ in ppm rel. to internal Me_4Si (= 0 ppm); digital resolution for coupling constants, 0.25 Hz/point; multiplicities of ^{13}C resonances from APT experiments; interchangeable assignments are starred (*). MS: *VG-70-250* or *VG ZAB*; NBA = 3-nitrobenzyl alcohol.

Biological Materials. The peptide angiotensin II was purchased from *Sigma Chemicals Ltd.*, St. Louis, USA, or from *Fluka AG*. [γ-^{32}P]ATP was from *Amersham*. The intracellular domain of the EGF-R (EGF-R ICD) and *c-src* kinases were expressed in Sf9 cells using recombinant baculoviruses and purified as previously described [19] [22]. Recombinant *v-abl* kinase was expressed in *E. coli* using vector pablHP. The fusion A-protein product encoded by this vector was affinity purified on *IgG-Affigel 10* as previously described [23].

4-(3-Hydroxypropyloxy)benzaldehyde (**3**). Following a known procedure [16], a mixture of 4-hydroxybenzaldehyde (**1**; 5.01 g, 41 mmol), K_2CO_3 (5.94 g, 43 mmol), 3-chloropropan-1-ol (**6**; 3.43 ml, 41 mmol), and DMF (10 ml) was heated to 80° for 24 h. After addition of H_2O (100 ml), the mixture was extracted twice with AcOEt (50 ml) and washed with 2N Na_2CO_3. The aq. layer was extracted with AcOEt and the combined org. phase washed with sat. NH_4Cl soln., dried, and evaporated: 7.19 g (98%) of clear red oil. Purification of 2.10 g of crude product by FC (200 g of silica gel, AcOEt/pentane 4:6→1:1) yielded 1.54 g of **3** (73% rel. to crude product) as colorless oily crystals with pink impurities. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.82 (*s*, CHO); 7.79 (*d*, J = 8.8, H–C(2), H–C(6)); 6.98 (*d*, J = 8.8, H–C(3), H–C(5)); 4.18 (*t*, J = 6.2, ArOCH_2); 3.86 (*t*, J = 6.0, CH_2OH); 2.98 (br. *s*, OH); 2.06 (*quint.*, J = 6.1, $CH_2CH_2CH_2$). ^{13}C-NMR (75 MHz, $CDCl_3$): 191.0 (CHO); 164.1 (C(4)); 132.0 (C(2), C(6)); 129.8 (C(1)); 114.8 (C(3), C(5)); 65.5 (ArOCH_2); 59.4 (CH_2OH); 31.9 ($CH_2CH_2CH_2$). EI-MS: (70 eV): 181 (4, $[M+1]^+$), 180 (39, M^+), 122 (51, $[OHC_6H_4CHO]^+$), 121 (100, $[OHC_6H_4CHO-H]^+$), 93 (9), 77 (7), 65 (16), 51 (7), 41 (11), 39 (13). CI-MS (NH_3): 198 (21, $[M+NH_4]^+$), 182 (11), 181 (100, $[M+H]^+$).

3-{4-[(E)-2-Nitroethenyl]phenyloxy}propan-1-ol (**11**). Following [18], a soln. of **3** (1.43 g, 7.9 mmol), $MeNO_2$ (515 μl, 9.51 mmol), and $MeNH_2$ (100 μl, 0.8 mmol) in EtOH (10 ml) was stirred at r.t. for 25 h. The turbid yellow suspension was evaporated *in vacuo* ($\leq 50°$) and dissolved in AcOEt and 2N HCl. The resulting emulsion was suction filtered, the org. layer of the filtrate washed with sat. NH_4Cl soln. (NaCl was added to improve phase separation), dried, and evaporated: 0.80 g of a yellow powder (**11**/**3** 78:22 (^{1}H-NMR)). FC (80 g of silica gel, AcOEt/$CHCl_3$ 1:1) yielded 252 mg (14%) of anal. pure **11**. Shining yellow powder. FT-IR (KBr): 3530 (OH), 3116 (arom. CH), 2947 and 2880 (aliph. CH), 1626, 1603, 1570, 1515, 1492, 1425, 1348, 1311, 1254, 1178, 1056, 998, 970, 829, 593, 526. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.96 (*d*, J = 13.7, CH=CHNO$_2$); 7.51 (*d*, J = 13.7, CH=CHNO$_2$); 7.49 (*d*, J = 8.8, H–C(3′), H–C(5′)); 6.96 (*d*, J = 8.7, H–C(2′), H–C(6′)); 4.18 (*t*, J = 6.0, ArOCH_2); 3.87 (*t*, J = 5.8, CH_2OH); 2.07 (*quint.*, J = 5.9, $CH_2CH_2CH_2$); 1.72 (*s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 162.3 (*s*, C(1′)); 139.0, 135.1 (2*d*, CH=$CHNO_2$); 131.2 (*d*, C(3′), C(5′)); 122.6 (*s*, C(4′)); 115.4 (*d*, C(2′), C(6′)); 65.6 (*t*, ArOCH_2); 59.8 (*t*, CH_2OH); 31.9 (*t*, $CH_2CH_2CH_2$). EI-MS (70 eV): 224 (2, $[M+1]^+$), 223 (18, M^+), 176 (6), 165 (7), 148 (6), 119 (23), 118 (100), 107 (43), 91 (36), 89 (45), 77 (14), 65 (35), 63 (41), 51 (21). Anal. calc. for $C_{11}H_{13}NO_4$ (223.23): C 59.19, H 5.87, N 6.27; found: C 59.13, H 5.60, N 6.17.

As a by-product of the filtering procedure, 0.59 g of a light-brown powder was isolated. Its low solubility in org. solvents and unusually broad ^{1}H-NMR signals indicate the formation of a polystyrene-type compound.

Methyl 3-{4-[(E)-2-Nitroethenyl]phenyloxy}propyl Pentanedioate (**15**). Following [14], TfOH (22 μl, 0.22 mmol) and methyl 5-chloro-5-oxopentanoate (**18**; 135 μl, 0.98 mmol) were added under Ar to a soln. of **11** (containing 6% of **3**; 218 mg, 0.98 mmol) in abs. CH_2Cl_2 (50 ml). After refluxing for 4 h, the mixture was cooled and CH_2Cl_2 (40 ml) added. The org. phase was washed successively with H_2O (25 ml) ice/sat. $NaHCO_3$ soln. (25 ml), and sat. NH_4Cl soln. (10 ml). The aq. layers were extracted with AcOEt and the combined org. phases dried and evaporated: 286 mg (83%) of anal. pure **15**. Yellow powder. FT-IR (KBr): 3103 (arom. CH), 2965 (aliph. CH), 1741 and 1720 (C=O), 1629, 1602, 1519, 1489, 1431, 1397, 1325, 1320, 1315, 1262, 1175, 1063, 1029, 971, 836, 590. ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): 8.11 (*s*, CH=CHNO$_2$); 7.82 (*d*, J = 8.8, H–C(3″), H–C(5″)); 7.04 (*d*, J = 8.8, H–C(2″), H–C(6″)); 4.18* (*t*, J = 6.5, ArOCH_2); 4.13* (*t*, J = 6.2, ArOCH$_2$CH$_2$CH_2); 3.58 (*s*, MeO); 2.35, 2.34

(2*t*, *J* = 7.3, CH_2(2), CH_2(4)); 2.05 (*quint.*, *J* = 6.3, $OCH_2CH_2CH_2O$); 1.77 (*quint.*, *J* = 7.3, CH_2(3)). ^{13}C-NMR (75 MHz, $(CD_3)_2SO$): 172.6, 172.2 (2 C=O); 161.5 (C(1″)); 139.1, 135.6 (*C*H=*C*HNO$_2$); 131.8 (C(3″), C(5″)); 122.6 (C(4″)); 115.0 (C(2″), C(6″)); 64.6 ($ArOCH_2$); 60.7 ($ArOCH_2CH_2CH_2$); 51.2 (MeO); 32.5, 32.3 (C(2), C(4)); 27.9 ($ArOCH_2CH_2$); 19.8 (C(3)). EI-MS (70 eV): 351 (0.1, M^+), 223 (0.8, [**11**]$^+$), 206 (9), 188 (10), 187 (100), 129 (31), 101 (35), 89 (4), 59 (28), 55 (17). CI-MS (NH_3): 369 (24, $[M + NH_4]^+$), 352 (37, $[M + H]^+$), 337 (27), 322 (100), 320 (34), 187 (54), 146 (55), 131 (25), 114 (89). Anal. calc. for $C_{17}H_{21}NO_7$ (351.35): C 58.11, H 6.02, N 3.99; found: C 58.07, H 5.85, N 4.11.

*3-{4-[(*E*)-2-Nitroethenyl]phenyloxy}propyl Hydrogen Pentanedioate* (**19**). Following [14], a soln. of **11** (222 mg, 1.0 mmol), glutaric anhydride (160 mg, 1.4 mmol), and 4-(dimethylamino)pyridine (16 mg, 0.1 mmol) in abs. $CHCl_3$ (10 ml) was stirred under Ar at r.t. for 19.5 h and then refluxed for 45.5 h to give a clear, dark-yellow soln. After addition of $CHCl_3$ (25 ml), the soln. was washed with 2N HCl (25 ml) and the org. phase dried and evaporated: 372 mg of yellow crude product. Purification by FC (37 g of silica gel, CH_2Cl_2/MeOH 19:1) yielded 268 mg (0.8 mmol, 80%) of **19** as a yellow powder and 66 mg (0.12 mmol, 6%) of bis{3-{4-[(*E*)-2-nitroethenyl]phenyloxy}propyl} pentanedioate.

19: FT-IR (KBr): 3500–2500 (COOH), 3106 (arom. CH), 2960 (aliph. CH), 1731 (C=O, ester), 1703 (C=O, acid), 1626, 1602, 1570, 1518, 1496, 1480, 1429, 1394, 1339, 1315, 1288, 1254, 1178, 1066, 1026, 970, 842, 828, 739, 589. ^{1}H-NMR (300 MHz, $CDCl_3$): 11.0–10.0 (br. *s*, COOH); 7.96 (*d*, *J* = 13.5, *CH*=CHNO$_2$); 7.52 (*d*, *J* = 13.5, CH=*CH*NO$_2$); 7.50 (*d*, *J* = 8.8, H–C(3″), H–C(5″)); 6.95 (*d*, *J* = 8.8, H–C(2″), H–C(6″)); 4.30 (*t*, *J* = 6.2, CH_2OCO); 4.11 (*t*, *J* = 6.1, $ArOCH_2$); 2.42 (2*t*, *J* = 7.3, CH_2(2), CH_2(4)); 2.15 (*quint.*, *J* = 6.2, $ArOCH_2CH_2$); 1.95 (*quint.*, *J* = 7.3, CH_2(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 179.0 (*s*, C=O, acid); 172.8 (*s*, C=O, ester); 162.1 (*s*, C(1″)); 139.0, 135.1 (2*d*, CH=CHNO$_2$); 131.2 (*d*, C(3″), C(5″)); 122.7 (*s*, C(4″)); 115.4 (*d*, C(2″), C(6″)); 64.7 (*t*, $ArOCH_2$); 61.1 (*t*, $ArOCH_2CH_2CH_2$); 33.1, 33.0 (2*t*, C(2), C(4)); 28.5 (*t*, $ArOCH_2CH_2$); 19.8 (*t*, C(3)). FAB-MS (NBA + KCl): 376 (25, $[M + K]^+$), 338 (43, $[M + H]^+$), 337 (15, M^+), 321 (5), 224 (37), 206 (20), 173 (100).

*Bis{3-{4-[(*E*)-2-Nitroethenyl]phenyloxy}propyl} Pentanedioate*: FT-IR (KBr): 3112 (arom. CH), 2950, 2925 and 2850 (aliph. CH), 1734 (C=O), 1631, 1602, 1572, 1516, 1500, 1338, 1310, 1255, 1174, 1053, 969, 820, 588, 520. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.96 (*d*, *J* = 13.5, 2 *CH*=CHNO$_2$); 7.51 (*d*, *J* = 13.5, 2 CH=*CH*NO$_2$); 7.50 (*d*, *J* = 8.6, 2 H–C(3″), 2 H–C(5″)); 6.94 (*d*, *J* = 8.9, 2 H–C(2″), 2 H–C(6″)); 4.28 (*t*, *J* = 6.3, 2 CH_2OCO); 4.10 (*t*, *J* = 6.1, 2 $ArOCH_2$); 2.39 (*t*, *J* = 7.4, CH_2(2), CH_2(4)); 2.14 (*quint.*, *J* = 6.2, 2 $ArOCH_2CH_2$); 1.95 (*quint.*, *J* = 7.2, CH_2(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.8 (*s*, 2 C=O); 162.1 (*s*, 2 C(1″)); 138.9, 135.1 (2*d*, 2 CH=CHNO$_2$); 131.2 (*d*, 2 C(3″), 2 C(5″)); 122.7 (*s*, 2 C(4″)); 115.4 (*d*, 2 C(2″), 2 C(6″)); 64.7 (*t*, 2 $ArOCH_2$); 61.1 (*t*, 2 $ArOCH_2CH_2CH_2$); 33.2 (*t*, C(2), C(4)); 28.5 (*t*, 2 $ArOCH_2CH_2$); 20.1 (*t*, C(3)). FAB-MS (NBA): 543 (4, $[M + H]^+$), 419 (5), 391 (16), 378 (9), 363 (5), 279 (12), 206 (14), 178 (7), 149 (99). FAB-MS (NBA + KCl): 581 (6, $[M + K]^+$), 543 (7, $[M + H]^+$), 429 (2), 391 (4), 378 (15), 363 (2), 335 (2), 317 (5), 279 (13), 206 (23), 178 (11), 149 (60).

*2′,3′-O-Isopropylideneadenosine 5′-{3-{4-[(*E*)-2-Nitroethenyl]phenyloxy}propyl Pentanedioate}* (**21**). Following [14], oxalyl chloride (280 µl, 3.2 mmol) and DMF (5 µl, 64 µmol) were added at 0° under Ar to a soln. of **19** (215 mg, 0.64 mmol) in abs. $CHCl_3$ (20 ml). The flask was purged with Ar and closed with a $CaCl_2$-drying tube, the ice-bath removed and the mixture stirred for 3 h 10 min and then evaporated. The resulting acyl chloride (yellow oil) in DMF (10 ml) was added dropwise to a soln. of 2′,3′-*O*-isopropylideneadenosine (198 mg, 1.0 mmol) and 4-(dimethylamino)pyridine (2.8 mg, 13 µmol) in DMF (5 ml) within 40 min. The mixture was stirred at r.t. for 2 h. After addition of H_2O, the mixture was extracted with $CHCl_3$ (50 ml) and the org. phase dried and evaporated: 343 mg of a clear, yellow oil. Purification by FC (40 g of silica gel, CH_2Cl_2/MeOH 19:1) gave 135 mg of a yellow oil, which was rechromatographed (15 g of silica gel, CH_2Cl_2/MeOH 100:0→90:10): 52 mg of yellow **21** with minor impurities. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.35, 7.89 (2*s*, H–C(2″), H–C(8″)); 7.98 (*d*, *J* = 13.5, *CH*=CHNO$_2$); 7.53 (*d*, *J* = 13.5, CH=*CH*NO$_2$); 7.49 (*d*, *J* = 8.7, H–C(3‴), H–C(5‴)); 6.93 (*d*, *J* = 8.8, H–C(2‴), H–C(6‴)); 6.10 (*d*, *J* = 2.0, H–C(1′)); 5.92 (br. *s*, NH_2); 5.48 (*dd*, *J* = 6.3, 1.9, H–C(2′)); 5.06 (*dd*, *J* = 6.3, 3.4, H–C(3′)); 4.47 (*m*, H–C(4′)); 4.36 (*dd*, *J* = 11.8, 4.5, 1 H–C(5′)); 4.27 (*t*, *J* = 6.3, CH_2(1‴)); 4.23 (*dd*, *J* = 11.8, 6.3, 1 H–C(5′)); 4.09 (*t*, *J* = 6.1, CH_2(3‴)); 2.31 (*m*, CH_2(2), CH_2(4)); 2.13 (*quint.*, *J* = 6.2, CH_2(2‴)); 1.89 (*quint.*, *J* = 7.3, CH_2(3)); 1.62, 1.40 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.7, 172.3 (2 C=O); 162.1 (C(1‴)); 155.5 (C(6″)); 153.0 (C(2″)); 149.3 (C(4″)); 139.9 (C(8″)); 138.9, 135.2 (*C*H=*C*HNO$_2$); 131.2 (C(3‴), C(5‴)); 122.8 (C(4‴)); 120.3 (C(5″)); 115.4 (C(2‴), C(6‴)); 114.5 (Me_2C); 91.1 (C(1′)); 85.0, 84.2, 81.7 (C(4′), C(3′), C(2′)); 64.7 (C(3‴)); 64.1 (C(5′)); 61.1 (C(1‴)); 33.1, 32.9 (C(2), C(4)); 28.5 (C(2‴)); 27.5, 25.4 (Me_2C); 19.9 (C(3)).

4-(2-Hydroxyethyloxy)benzaldehyde (**2**). A mixture of 4-hydroxybenzaldehyde (**1**; 5.00 g, 41 mmol), K_2CO_3 (6.03 g, 43 mmol), and 2-chloroethanol (**5**; 2.74 ml, 41 mmol) in DMF (10 ml) was heated to 80° for 22 h, whereupon H_2O/AcOEt 1:1 (100 ml) were added. The aq. layer was separated and extracted with AcOEt (50 ml) and the combined org. phase washed with 1N NaOH (2 × 25 ml) and sat. NH_4Cl soln. (25 ml), dried, and

evaporated: 4.03 g (59%) of **2**. Red oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.85 (*s*, CHO); 7.81 (*d*, *J* = 8.6, H–C(2), H–C(6)); 7.01 (*d*, *J* = 8.7, H–C(3), H–C(5)); 4.17* (*t*, *J* = 4.6, $ArOCH_2$); 4.01* (*t*, *J* = 4.6, CH_2OH); 3.18 (br. *s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 191.0 (CHO); 163.9 (C(4)); 132.0 (C(2), C(6)); 130.0 (C(1)); 114.9 (C(3), C(5)); 69.7 ($ArOCH_2$); 61.0 (CH_2OH).

*2-{4-[(*E*)-2-Nitroethenyl]phenyloxy}ethan-1-ol* (**10**). A mixture of **2** (1.09 g, 6.6 mmol), $MeNO_2$ (15 ml), and aniline (60 µl, 0.66 mmol) was heated to 80° for 30 h 40 min. Evaporation of the mixture yielded 1.28 g (100%) of an orange-yellow oily solid. Chromatography (125 g of silica gel, AcOEt/pentane 1:1) yielded 294 mg of **10** as a yellow powder (containing, according to NMR, 50% of **2**). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.97 (*d*, *J* = 13.5, CH=$CHNO_2$); 7.51 (*d*, *J* = 13.5, CH=$CHNO_2$); 7.50 (*d*, *J* = 8.8, H–C(3′), H–C(5′)); 6.97 (*d*, *J* = 8.8, H–C(2′), H–C(6′)); 4.15* (*t*, *J* = 4.2, $ArOCH_2$); 4.01* (*t*, *J* = 3.8, CH_2OH); 2.37 (br. *s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 161.8 (*s*, C(1′)); 138.8, 135.1 (2*d*, CH=$CHNO_2$); 131.1 (*d*, C(3′), C(5′)); 122.8 (*s*, C(4′)); 115.3 (*d*, C(2′)); C(6′)); 69.5 (*t*, $ArOCH_2$); 61.1 (*t*, CH_2OH).

*Methyl 2-{4-[(*E*)-2-Nitroethenyl]phenyloxy}ethyl Pentanedioate* (**14**). Following [14], **18** (210 µl, 1.5 mmol) and TfOH (30 µl, 0.3 mmol) were added under Ar to **10** (containing *ca.* 50% of **2**; 294 mg, 1.5 mmol) in abs. $CHCl_3$ (30 ml). The mixture was refluxed for 1 h 40 min. After addition of ice and $CHCl_3$, the org. layer was extracted with ice/sat. $NaHCO_3$ soln., washed with sat. NH_4Cl soln., dried, and evaporated. The crude product (0.55 g) was purified by FC (60 g of silica gel, AcOEt/pentane 1:1): 178 mg (0.53 mmol, 70% rel. to **10**) of **14**. Yellow solid. FT-IR (KBr): 3116 (arom. CH), 2952 (aliph. CH), 1733 (C=O), 1690, 1628, 1603, 1575, 1514, 1490, 1428, 1384, 1342, 1312, 1259, 1198, 1169, 1102, 1061, 968, 937, 823. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.95 (*d*, *J* = 13.5, CH=$CHNO_2$); 7.54 (*d*, *J* = 13.6, CH=$CHNO_2$); 7.53 (*d*, *J* = 8.8, H–C(3″), H–C(5″)); 6.98 (*d*, *J* = 8.8, H–C(2″), H–C(6″)); 4.47* (*t*, *J* = 4.7, $ArOCH_2$); 4.25* (*t*, *J* = 4.8, $ArOCH_2CH_2$); 3.66 (*s*, MeO); 2.44* (*t*, *J* = 7.2, CH_2(2)); 2.40* (*t*, *J* = 7.0, CH_2(4)); 1.96 (*quint.*, *J* = 7.3, CH_2(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.0, 172.5 (2*s*, 2 C=O); 161.6 (*s*, C(1″)); 138.6, 135.1 (2*d*, CH=$CHNO_2$); 131.1 (*d*, C(3″), C(5″)); 122.9 (*s*, C(4″)); 115.3 (*d*, C(2″), C(6″)); 66.1 (*t*, $ArOCH_2$); 62.3 (*t*, $ArOCH_2CH_2$); 51.5 (*q*, MeO); 33.0, 32.9 (2*t*, C(2), C(4)); 20.0 (*t*, C(3)). EI-MS (70 eV): 337 (1, M^+), 263 (2), 192 (5), 174 (9), 173 (100), 129 (4), 101 (11), 99 (53). CI-MS (NH_3): 357 (4), 356 (16), 355 (87, $[M + NH_4]^+$), 338 (9, $[M + H]^+$), 324 (7), 323 (16), 322 (11), 309 (19), 308 (100), 173 (24). Anal. calc. for $C_{16}H_{19}NO_7$ (337.33): C 56.97, H 5.68, N 4.15; found: C 56.70, H 5.49, N 4.07.

4-(4-Hydroxybutyloxy)benzaldehyde (**4**). To a soln. of **1** (10.00 g, 82 mmol) in DMF (10 ml), a suspension of K_2CO_3 (11.89 g, 86 mmol) and KI (13.60 g, 82 mmol; both ground to a fine powder) in DMF (40 ml) was added under mechanical stirring (→red and warming up to 40°). After addition of 4-chlorobutan-1-ol (**7**; 8.15 ml, 82 mmol) in DMF (50 ml), the suspension was stirred at 82–93° for 93.5 h. AcOEt and H_2O were added to the mixture. The org. phase was washed with 4 × 2N NaOH, sat. NH_4Cl soln., 2N HCl, and 2 × sat. NH_4Cl soln., dried, and evaorated: 3.39 g (21%) of **4** as a reddish clear oil (NMR: small amount of **1** present). ^{1}H-NMR (300 MHz, $CDCl_3$): 9.85 (*s*, CHO); 7.81 (*d*, *J* = 8.9, H–C(2), H–C(6)); 6.98 (*d*, *J* = 8.8, H–C(3), H–C(5)); 4.08 (*t*, *J* = 6.3, $ArOCH_2$); 3.73 (*t*, *J* = 6.3, CH_2OH); 2.62 (br. *s*, OH); 1.92, 1.76 (2*m*, $ArOCH_2CH_2CH_2$). ^{13}C-NMR (75 MHz, $CDCl_3$): 190.7 (CHO); 163.9 (C(4)); 131.9 (C(2), C(6)); 129.6 (C(1)); 114.6 (C(3), C(5)); 68.1 ($ArOCH_2$); 62.1 (CH_2OH); 25.6 ($ArOCH_2CH_2$); 29.1 (CH_2CH_2OH).

*4-{4-[(*E*)-2-Nitroethenyl]phenyloxy}butan-1-ol* (**12**). Aniline (1.63 g, 17 mmol) was added to a soln. of **4** (3.39 g, 17 mmol) in $MeNO_2$ (25 ml) and heated to 80° for 15 h 45 min. AcOEt was added to the mixture and the org. phase washed with 2 × 2N HCl, sat. NH_4Cl soln., 2N NaOH, and sat. NH_4Cl soln., dried, and evaporated. An attempt to recrystallize the crude product (5.13 g) resulted in a moist yellow product (NMR: small impurities present). Purification by chromatography (*Merck* silica gel, *Büchi* MPLC, 10 bar, AcOEt/pentane 50:50→100:0) yielded 2.11 g (8.9 mmol, 52%) of **12** as shining, yellow, cotton-like crystals (NMR: presence of small amounts of aniline and decomposition products probably due to the purification by chromatography). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.98 (*d*, *J* = 13.5, CH=$CHNO_2$); 7.52 (*d*, *J* = 13.5, CH=$CHNO_2$); 7.50 (*d*, *J* = 8.8, H–C(3′), H–C(5′)); 6.95 (*d*, *J* = 8.9, H–C(2′), H–C(6′)); 4.07 (*t*, *J* = 6.2, $ArOCH_2$); 3.72 (*t*, *J* = 6.3, CH_2OH); 1.93, 1.77 (2*m*, $CH_2CH_2CH_2OH$); 1.44 (*s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 162.4 (C(1′)); 139.1, 134.9 (CH_2=$CHNO_2$); 131.2 (C(3′), C(5′)); 122.5 (C(4′)); 115.4 (C(2′), C(6′)); 68.1 ($ArOCH_2$); 62.3 (CH_2OH); 25.6 ($ArOCH_2CH_2$); 29.2 (CH_2CH_2OH).

*Methyl 4-{4-[(*E*)-2-Nitroethenyl]phenyloxy}butyl Pentanedioate* (**16**). Under Ar, **18** (580 µl, 4.2 mmol) and TfOH (37 µl, 0.42 mmol) were added to a soln. of **12** (1.00 g, 4.2 mmol) in abs. $CHCl_3$ (25 ml). The dark red soln. was refluxed for 7.5 h. After addition of ice and $CHCl_3$, the org. phase was washed with sat. $NaHCO_3$ soln., dried, and evaporated. The crude product (1.46 g, 95%, light brown powder) was recrystallized from MeOH: 0.91 g (60%) of a dark yellow powder. FT-IR (KBr): 3098 (arom. CH), 2952 and 2860 (aliph. CH), 1725 (C=O), 1630, 1601, 1572, 1522, 1492, 1476, 1432, 1341, 1316, 1283, 1256, 1195, 1180, 1065, 1019, 970, 839, 809, 753, 592. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.96 (*d*, *J* = 13.5, CH=$CHNO_2$); 7.52 (*d*, *J* = 13.5, CH=$CHNO_2$); 7.50 (*d*, *J* = 8.8,

H–C(3″), H–C(5″)); 6.94 (*d*, *J* = 8.8, H–C(2″), H–C(6″)); 4.16* (*t*, *J* = 6.2, ArOCH_2); 4.05* (*t*, *J* = 5.9, CH_2OCO); 3.67 (*s*, MeO); 2.38 (*t*, *J* = 7.4, CH_2(2), CH_2(4)); 1.96 (*quint.*, *J* = 7.4, CH_2(3)); 1.86 (*m*, ArOCH$_2$CH_2CH_2). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.3, 172.9 (2 C=O); 162.3 (C(1″)); 139.0, 135.1 (*C*H=*C*HNO$_2$); 131.2 (C(3″), C(5″)); 122.6 (C(4″)); 115.4 (C(2″), C(6″)); 67.7 (ArO*C*H$_2$); 63.9 (*C*H$_2$OCO); 51.6 (MeO); 33.3, 33.1 (C(2), C(4)); 25.7, 25.4 (ArOCH$_2$*C*H$_2$*C*H$_2$); 20.2 (C(3)). EI-MS (70 eV): 365 (11, M^+), 220 (22), 201 (34), 178 (12), 165 (6), 148 (5), 129 (100), 118 (15), 115 (13), 101 (46), 89 (6), 71 (6), 59 (24), 55 (64). Anal. calc. for $C_{18}H_{23}NO_7$ (365.38): C 59.17, H 6.34, N 3.83; found: C 59.08, H 6.07, N 3.88.

4-(Hydroxymethyl)benzaldehyde (**9**). Following [17], 3.630 l of H_2 were added under normal pressure and vigorous shaking at r.t. to a suspension of 20.0 g (150 mmol) of benzene-1,4-dicarbaldehyde (**8**) and 0.1 g of 10% Pd/C (94 μmol Pd) in 100 ml of 80% EtOH. The mixture was filtered over *Celite*, washed with EtOH (30 ml), the solvent evaporated, and the residue dissolved in AcOEt. The org. phase was washed with H_2O (20 ml) and sat. NH_4Cl soln. (20 ml), dried and evaporated: 17.59 g of colorless crystals containing 3% of **8** and 3% of 1,4-bis-(hydroxymethyl)benzene (by ^{1}H-NMR). Thereof, 5 g were purified by chromatography (500 g silica gel, CH_2Cl_2/MeOH 95:5): 4.24 g (85%) of pure **9** [24] (overall yield 74%). ^{1}H-NMR (300 MHz, $CDCl_3$): 9.94 (*s*, CHO); 7.82 (*d*, *J* = 8.2, H–C(2), ,H–C(6)); 7.49 (*d*, *J* = 8.0, H–C(3), H–C(5)); 4.76 (*s*, CH_2O); 3.04 (br. *s*, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 192.3 (*s*, CHO); 148.1 (*s*, C(4)); 135.5 (*s*, C(1)); 130.0 (*d*, C(2), C(6)); 127.0 (*d*, C(3), C(5)); 64.4 (*t*, CH_2).

4-[(E)-2-Nitroethenyl]benzene-1-methanol (**13**). A soln. of **9** (7.40 g, 54 mmol) and aniline (5 ml, 54 mmol) in $MeNO_2$ (35 ml) was heated to 90° for 70 h. After addition of AcOEt (100 ml), the mixture was washed with 2N HCl and sat. NH_4Cl soln. and the org. phase dried and evaporated: 11.93 g of an orange-brown solid. Large amounts of reasonably pure product were obtained by recrystallizing the crude product twice from AcOEt. Purification of a sample by chromatography (*Merck Florisil*, Et_2O/pentane 2:1) gave anal. pure **13** in 62% yield as air-sensitive, strongly mucuous membrane-irritating yellow needles. M.p. 114.0–115.5°. FT-IR (KBr): 3520 (br., OH), 3114 and 3044 (arom. CH), 2906 (aliph. CH), 1630, 1607, 1568, 1496, 1414, 1338, 1265, 1201, 1181, 1022, 968, 828, 787, 727. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.01 (*d*, *J* = 13.7, C*H*=CHNO$_2$); 7.59 (*d*, *J* = 13.5, CH=C*H*NO$_2$); 7.56 (*d*, *J* = 8.0, H–C(3), H–C(5)); 7.46 (*d*, *J* = 8.2, H–C(2), H–C(6)); 4.77 (*d*, *J* = 5.5, CH_2OH); 1.80 (*t*, *J* = 5.8, OH). ^{13}C-NMR (75 MHz, $CDCl_3$): 145.4 (C(1)); 138.7, 137.0 (*C*H=*C*HNO$_2$); 129.4* (C(3), C(5)); 129.3 (C(4)); 127.5* (C(2), C(6)); 64.6 (CH_2OH). EI-MS (70 eV): 180 (7), 179 (71, $[M]^+$), 161 (6), 148 (34, $[M - CH_2OH]^+$), 133 (12), 132 (64), 131 (41), 119 (15), 115 (27), 103 (82), 91 (51), 77 (100), 65 (18), 63 (21), 51 (38). Anal. calc. for $C_9H_9NO_3$ (179.18): C 60.33, H 5.06, N 7.82; found: C 60.21, H 4.80, N 7.94.

4-[(E)-Nitroethenyl]benzyl Hydrogen Pentanedioate (**20**). A soln. of impure **13** (11 g, *ca.* 38 mmol), glutaric anhydride (6.24 g, 54 mmol), and 4-(dimethylamino)pyridine (0.67 g, 5.4 mmol) in abs. $CHCl_3$ (130 ml), was stirred at r.t. under Ar for 22 h. The mixture was washed with 2N HCl and sat. NH_4Cl soln., dried, and evaporated: 14.45 g of crude product. Purification by chromatography (silica gel, CH_2Cl_2/MeOH 95:5) gave 6.98 g (63%) of **20**. Yellow crystals. M.p. 99.0–100.5°. FT-IR (KBr): 3435 (br., COOH), 3109 (arom. CH), 2942 (aliph. CH), 1745 (C=O, ester), 1702 (C=O, acid), 1634, 1610, 1570, 1508, 1410, 1344, 1302, 1260, 1175, 1153, 1002, 969, 954, 825. ^{1}H-NMR (300 MHz, $CDCl_3$): 10.5–9.5 (br. *s*, COOH); 8.00 (*d*, *J* = 13.5, C*H*=CHNO$_2$); 7.59 (*d*, *J* = 13.6, CH=C*H*NO$_2$); 7.55* (*d*, *J* = 8.0, H–C(3′), H–C(5′)); 7.43* (*d*, *J* = 8.0, H–C(2′), H–C(6′)); 5.16 (*s*, CH_2(7′)); 7.49* (*t*, *J* = 7.4, CH_2(2)); 2.45* (*t*, *J* = 7.1, CH_2(4)); 1.99 (*quint.*, *J* = 7.2, CH_2(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 178.8 (C(5)); 172.5 (C(1)); 140.2 (C(1′)); 138.4, 137.4 (*C*H=*C*HNO$_2$); 129.9 (C(4′)); 129.3* (C(3′), C(5′)); 128.8* (C(2′), C(6′)); 65.4 (C(7′)); 33.1* (C(2)); 32.9* (C(4)); 19.8 (C(3)). FAB-MS (NBA): 316 (10, $[M + Na]^+$), 294 (59, $[M + H]^+$), 179 (10, **[13]**$^+$), 163 (31), 162 (100, $[O_2NCH{=}CHC_7H_6]^+$), 146 (9), 145 (12), 131 (7), 117 (8), 116 (8), 115 (50), 91 (8), 87 (10), 74 (4), 71 (4), 55 (10), 43 (11). Anal. calc. for $C_{14}H_{15}NO_6$ (293.28): C 57.34, H 5.16, N 4.78; found: C 57.07, H 5.05, N 4.72.

Methyl 4-[(E)-Nitroethenyl]benzyl Pentanedioate (**17**). Following [14], **18** (1.17 ml, 8.1 mmol) and TfOH (130 μl, 1.5 mmol) were added under Ar at 0° to a soln. of impure **13** (1.52 g, *ca.* 5.3 mmol) in abs. CH_2Cl_2 (50 ml). The mixture was refluxed for 7.5 h and then washed with H_2O (50 ml) and ice/sat. $NaHCO_3$ soln., dried, and evaporated: 2.52 g of dark yellow crude product. Purification by chromatography (250 g of silica gel, pentane/AcOEt 3:1) gave 0.52 g (32%) of **17**. A sample was recrystallized twice from MeOH: shining yellow plates. M.p. 73.0–74.5°. FT-IR (KBr): 3097 (arom. CH), 3032, 2951 (aliph. CH), 1732 (C=O), 1625, 1613, 1499, 1453, 1415, 1341, 1320, 1270, 1176, 1083, 987, 972, 817. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.00 (*d*, *J* = 13.7, C*H*=CHNO$_2$); 7.59 (*d*, *J* = 13.7, CH=C*H*NO$_2$); 7.56* (*d*, *J* = 8.4, H–C(3′), H–C(5′)); 7.43* (*d*, *J* = 8.3, H–C(2′), H–C(6′)); 5.16 (*s*, CH_2(7′)); 3.68 (*s*, MeO); 2.47* (*t*, *J* = 7.4, CH_2(2)); 2.40* (*t*, *J* = 7.3, CH_2(4)); 1.99 (*quint.*, *J* = 7.2, CH_2(3)). ^{13}C-NMR (75 MHz, $CDCl_3$): 173.2* (*s*, C(5)); 172.5* (*s*, C(1)); 140.2 (*s*, C(1′)); 138.4, 137.3 (2*d*, *C*H=*C*HNO$_2$); 129.8 (*s*, C(4′)); 129.3* (*d*, C(3′), C(5′)); 128.7* (*d*, C(2′), C(6′)); 65.3 (*t*, C(7′)); 51.6 (*q*, MeO); 33.2* (*t*, C(2)); 33.0* (*t*, C(4)); 20.1 (*t*, C(3)). CI-MS (NH_3): 326 (12), 325 (71, $[M + NH_4]^+$), 308 (19, $[M + H]^+$), 293 (19), 278 (21), 179

(5, **[13]**$^+$), 165 (9), 164 (100), 148 (9), 147 (20), 146 (18), 134 (26), 133 (8), 132 (62), 131 (9), 130 (9), 114 (4). Anal. calc. for $C_{15}H_{17}NO_6$ (307.30): C 58.63, H 5.58, N 4.56; found: C 58.72, H 5.69, N 4.43.

*2′,3′-O-Isopropylideneadenosine 5′-{4-[(*E*)-2-Nitroethenyl]benzyl Pentanedioate}* (**22**). Following [14], DMF (32 µl, 0.4 mmol) and **20** (0.67 g, 2.3 mmol) were added to oxalyl chloride (9 ml) at 0° under Ar. The mixture was stirred at r.t. for 2.5 h and evaporated under high vacuum. The acyl chloride obtained was dissolved in abs. DMF (4 ml) and added under Ar at 0° to a soln. of 2′,3′-*O*-isopropylideneadenosine (0.70 g, 2.3 mmol) and 4-(dimethylamino)pyridine (8.5 mg, 69 µmol) in abs. DMF (3 ml) within 5 min. The mixture was stirred for 3 h, AcOEt (80 ml) added, the org. phase washed with H_2O and ice/sat. $NaHCO_3$ soln. and evaporated, the residue dissolved in acetone (50 ml), and the soln. filtered, dried, and evaporated: 0.84 g of yellow crude product. Purification by chromatography (90 g of silica gel, AcOEt/acetone 2:1) gave 211 mg (16%) of **22**. Yellow amorphous solid. M.p. 45–60°. FT-IR (KBr): 3435 and 3350 (br., NH_2), 3177 and 3120 (arom. CH), 2986 and 2940 (aliph. CH), 1737 (C=O), 1636, 1598, 1522, 1375, 1340, 1207, 1160, 1105, 1076, 968, 869. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.34 (*s*, H–C(8″)); 7.98 (*d*, *J* = 13.7, C*H*=CHNO$_2$); 7.88 (*s*, H–C(2″)); 7.59 (*d*, *J* = 13.7, CH=C*H*NO$_2$); 7.53* (*d*, *J* = 8.3, H–C(3‴), H–C(5‴)); 7.40* (*d*, *J* = 8.2, H–C(2‴), H–C(6‴)); 6.10 (*d*, *J* = 1.9, H–C(1′)); 5.99 (br. *s*, NH_2); 5.49 (*dd*, *J* = 6.3, 2.0, H–C(2′)); 5.14 (*s*, CH_2(7‴)); 5.07 (*dd*, *J* = 6.3, 3.3, H–C(3′)); 4.46 (*m*, H–C(4′)); 4.37 (*dd*, 1 H, *J* = 11.8, 4.4, H–C(5′)); 4.24 (*dd*, 1 H, *J* = 11.8, 6.3, H–C(5′)); 2.41 (*t*, *J* = 7.3, CH_2(2)); 2.32 (*td*, *J* = 7.3, 2.3, CH_2(4)); 1.92 (*quint.*, *J* = 7.3, CH_2(3)); 1.62, 1.40 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.5, 172.3 (C(1), C(5)); 155.7 (C(6″)); 153.2 (C(2″)); 149.3 (C(4″)); 140.3 (C(1‴)); 139.8 (C(8″)); 138.4, 137.4 (*C*H=*C*HNO$_2$); 129.9 (C(4‴)); 129.3* (C(3‴), C(5‴)); 128.8* (C(2‴), C(6‴)); 120.3 (C(5″)); 114.6 (Me_2*C*); 91.0 (C(1′)); 85.0, 84.2, 81.7 (C(2′), C(3′), C(4′)); 65.4 (C(7‴)); 64.1 (C(5′)); 33.1, 32.9 (C(2), C(4)); 27.2, 25.4 (*Me*$_2$C); 19.9 C(3)). FAB-MS (NBA): 583 (50, [*M* + H]$^+$), 551 (20), 523 (20), 496 (15), 469 (15), 447 (50), 413 (10), 369 (15), 281 (20), 167 (30), 136 (100).

*Adenosine 5′-{4-[(*E*)-Nitroethenyl]benzyl Pentanedioate}* (**23**). A mixture of **22** (84 mg, 0.15 mmol) and 90% CF_3COOH (10 ml) was stirred at r.t. for 40 min [25]. Evaporation under high vacuum gave a yellow oily residue which was dissolved in AcOEt (50 ml). The soln. was washed with H_2O, dried, and evaporated: 75 mg of a yellow solid. Purification by chromatography (20 g of silica gel, AcOEt/acetone 2:1) gave 44 mg (56%) of **23**. Yellow, cotton-like solid. M.p. 50–85°. FT-IR (KBr): 3500–2500 (OH, NH_2), 3100 (arom. CH), 2940 (aliph. CH), 1736 (C=O), 1637, 1605, 1522, 1510, 1420, 1340, 1181, 970, 819. ^{1}H-NMR (400 MHz, $(CD_3)_2CO$): 8.21, 8.19 (2*s*, H–C(8″), H–C(2″)); 8.09, 7.97 (2*d*, *J* = 13.7, C*H*=C*H*NO$_2$); 7.82* (*d*, *J* = 8.4, H–C(3‴), H–C(5‴)), 7.50* (*d*, *J* = 8.4, H–C(2‴), H–C(6‴)); 6.72 (br. *s*, NH_2); 6.05 (*d*, *J* = 4.4, H–C(1′)); 5.18 (*s*, CH_2(7‴)); 4.87 (*t*, *J* = 4.9, H–C(2′)); 4.54 (*t*, *J* = 5.1, H–C(3′)); 4.42* (*dd*, 1 H, *J* = 11.9, 3.7, H–C(5′)); 4.33* (*dd*, 1 H, *J* = 11.9, 5.3, H–C(5′)); 4.26 (*m*, H–C(4′)); 2.95 (br. *s*, OH–C(2′), OH–C(3′)); 2.47* (*t*, *J* = 7.5, CH_2(2)); 2.43* (*t*, *J* = 7.7, CH_2(4)); 1.91 (*quint.*, *J* = 7.3, CH_2(3)). ^{13}C-NMR (101 MHz, $(CD_3)_2CO$): 173.1 (C(1), C(5)); 157.1 (C(6″)); 153.6 (C(2″)); 150.5 (C(4″)); 141.7 (C(8″)); 140.6 (C(1‴)); 139.2, 138.8 (*C*H=*C*HNO$_2$); 131.0 (C(4‴)); 130.5* (C(3‴), C(5‴)); 129.4* (C(2‴), C(6‴)); 120.3 (C(5″)); 89.9 (C(1′)); 83.0 (C(4′)); 74.8* (C(2′)); 71.8* (C(3′)); 65.8 (C(7‴)); 64.6 (C(5′)); 33.5, 33.4 (C(2), C(4)); 20.9 (C(3)). FAB-MS (NBA): 543 (15, [*M* + H]$^+$), 527 (10), 498 (10), 469 (15), 447 (40), 399 (25), 250 (10), 167 (20), 154 (70).

Kinase Assays. Determination of EGF-R ICD kinase activity was performed according to [26], using angiotensin II as substrate. All compounds were dissolved in DMSO and diluted with H_2O giving a final concentration of 1% in the assay. Genistein and erbstatin [27] served as references.

c-src Tyrosine protein kinase activity was assayed using polymer poly Glu, Tyr or angiotensin II as phosphate acceptor substrate. The reaction mixtures (50 µl) contained 20 mM *Tris*·HCl pH 7.5, 10 mM $MgCl_2$, 20 µM [γ-^{32}P]ATP (0.1 Ci/mmol), 100 ng of enzyme protein, and 25 µg/ml of polymeric substrate in 1 mM angiotensin II. Reactions were carried out at 20° for 10–15° min and terminated by adding 10 µl of ice-cold 0.5% H_3PO_4 soln. Aliquots of the mixture were spotted onto *P81* paper and processed according to [28] [29].

v-abl Kinase was assayed according to [29] using 1 mM [Val^5]angiotensin II and 10 mM [γ-^{32}P]ATP (0.33 Ci/mmol) as substrates.

Activity determinations of PKC_z using histone III-S (0.2 mg/ml) as substrate were performed according to [28].

IC_{50} Values were defined as the inhibitor concentrations that resulted in a 50% inhibition of substrate phosphorylation by the enzyme compared to the control experiment in the absence of inhibitor.

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